

11/11/96
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**NASA Progress Report
NAG-2-769
"An Evaluation of
Collagen Metabolism in
Non-Human Primates Associated With
the Bion Space Program-Markers of
Urinary Collagen Turnover and
Muscle Tissue Collagen Types"**

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10/7/96

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RESULTS

Humerus Biochemistry

Means \pm standard error for biochemical variables measured on the left humerus are shown in Table 1. No significant differences at $p < 0.05$ were found between groups when the following variables were measured: density, HYP, total collagen per mm^3 bone, phosphorus concentration, total phosphorus per mm^3 bone, calcium concentration, calcium to collagen ratio, and calcium to phosphorus weight ratio. The R+ML (66 d old) rodents showed a significantly greater amount of HP and total crosslinks than did their younger (38 d and 52 d old) counterparts. In addition, the R+ML F group had a significantly greater amount of LP crosslinks than both the R+O FC and L+O basal groups. The R+ML FC group exhibited a greater amount of calcium per mm^3 and total mineral compared to R+O FC. No significant differences were found between treatment groups of animals of similar age. Recovery of HYP for this study was 100%.

Tibia Biochemistry

Means \pm standard error for biochemical parameters examined on the left tibia are displayed in Table 2. No significant differences at $p < 0.05$ were found between groups when the following variables were analyzed: density, HYP, total collagen per mm^3 bone, phosphorus concentration, calcium concentration, total calcium per mm^3 bone, calcium to collagen ratio, calcium to phosphorus weight ratio, and total mineral content. The R+ML (66 d old) rodents exhibited a significantly greater amount of HP and total crosslinks, than rats 28 d younger (38 d old). The R+ML F and FC groups had significantly greater phosphorus per mm^3 than the L+O basal group. The amount of LP per mole of collagen in 52 d old (R+O) F rats was significantly greater than the R+O FC rats housed in similar size cages. No additional significant differences were noted among the variables between treatment groups of animals of similar age.

Collagen Accumulation

Means \pm standard error are shown for the humerus and tibia in Table 3. New and old collagen present are expressed as percentages of the total collagen content in the bone sample. No significant differences were found between groups at $p < 0.05$.

Urine Biochemistry

Means \pm standard error are shown in Figure 1 and Appendices F through M. Urine data was only available for the first four days following the landing of the shuttle. The F group had consistently lower specific gravities than the VC group. The creatinine concentration in the F group was also significantly less than that found in the VC group at most time points, however, the FC and VC groups did not differ from one another. Data within the F group fluctuated, in that it decreased in HYP during the first four days following landing, this fluctuation was slightly greater than that found within other treatment groups. The VC group was significantly greater in HYP than both the FC and F groups during the last two days of urine collection. HP values in the VC group were significantly greater than both the FC and F groups throughout days 11/03, 11/04 and 11/05. Both FC and F groups had decreasing HP, LP and total crosslink values during the initial 2 to 3 d following landing, respectfully. These groups then increased in HP. FC and F groups were not significantly different from one another in HP, LP or total crosslinks normalized to creatinine at 11/02 and 11/05.

Table 1

Humerus Biochemistry

	L+O		R+O			R+ML		
	Basal		VC	FC	F	VC	FC	F
Density (g/mL)	1.78 ± 0.05		1.77 ± 0.06	1.72 ± 0.03	1.79 ± 0.05	1.82 ± 0.07	1.87 ± 0.04	1.84 ± 0.04
µg HYP /mg dry wt.	24.23 ± 0.68		24.47 ± 1.63	25.11 ± 0.81	25.68 ± 0.81	26.12 ± 0.25	24.99 ± 0.87	26.21 ± 0.24
moles HP /mole collagen	0.11 ± 0.004 ^c		0.12 ± 0.01 ^{bc}	0.12 ± 0.001 ^{bc}	0.12 ± 0.004 ^c	0.17 ± 0.002 ^a	0.16 ± 0.01 ^a	0.15 ± 0.01 ^{ab}
moles LP /mole collagen	0.11 ± 0.01 ^{bc}		0.11 ± 0.01 ^{abc}	0.11 ± 0.01 ^{bc}	0.12 ± 0.001 ^{abc}	0.12 ± 0.01 ^{abc}	0.13 ± 0.01 ^{ab}	0.14 ± 0.01 ^a
moles x-links / mole collagen	0.22 ± 0.01 ^c		0.24 ± 0.02 ^{bc}	0.23 ± 0.01 ^c	0.24 ± 0.005 ^c	0.29 ± 0.01 ^{ab}	0.29 ± 0.01 ^a	0.29 ± 0.01 ^{ab}
Total µg Collagen / mm ³	307.84 ± 15.08		306.80 ± 18.89	308.49 ± 13.05	328.24 ± 16.89	339.90 ± 14.88	333.68 ± 11.92	344.89 ± 8.30
µg P / mg dry wt	222.57 ± 9.27		220.75 ± 2.02	236.31 ± 2.44	234.05 ± 8.00	229.67 ± 1.55	237.32 ± 3.18	241.66 ± 2.59
Total µg P / mm ³	394.62 ± 17.66		389.63 ± 13.12	405.88 ± 6.89	424.89 ± 21.99	418.42 ± 17.94	444.07 ± 11.50	445.68 ± 14.05
µg Ca ⁺⁺ / mg dry wt	150.77 ± 7.12		160.15 ± 4.91	140.38 ± 16.00	169.83 ± 12.08	159.07 ± 8.34	185.22 ± 21.45	169.05 ± 6.03
Total µg Ca ⁺⁺ / mm ³	268.11 ± 15.70 ^{ab}		281.87 ± 8.82 ^{ab}	239.39 ± 25.88 ^b	303.94 ± 23.97 ^{ab}	290.55 ± 21.16 ^{ab}	346.88 ± 42.24 ^a	311.57 ± 13.40 ^{ab}
Total µg Ca ⁺⁺ / Total µg Collagen	0.87 ± 0.04		0.94 ± 0.08	0.79 ± 0.09	0.92 ± 0.05	0.85 ± 0.04	1.04 ± 0.10	0.90 ± 0.03
Ca ⁺⁺ to P weight ratio	0.68 ± 0.04		0.73 ± 0.02	0.59 ± 0.07	0.77 ± 0.03	0.69 ± 0.04	0.78 ± 0.09	0.70 ± 0.03
Total mineral (µg/mm ³)	662.74 ± 28.93 ^{ab}		671.50 ± 19.16 ^{ab}	645.27 ± 23.26 ^b	658.02 ± 96.45 ^{ab}	708.97 ± 37.00 ^{ab}	790.95 ± 45.62 ^a	757.25 ± 23.01 ^{ab}

Values are means ± standard error. Groups which do not share a similar letter were found significantly different at p<0.05.

Table 2

Tibia Biochemistry

	L+O		R+O		R+ML	
	Basal		VC	FC	VC	FC
Density (g/mL)	1.71 ± 0.06		1.77 ± 0.02	1.88 ± 0.04	1.83 ± 0.04	1.89 ± 0.04
µg HYP /mg dry wt.	29.98 ± 3.95		25.68 ± 0.37	26.11 ± 0.67	24.42 ± 0.70	24.88 ± 0.40
moles HP /mole collagen	0.10 ± 0.01 ^c		0.14 ± 0.002 ^{ab}	0.14 ± 0.01 ^b	0.18 ± 0.004 ^a	0.17 ± 0.01 ^a
moles LP / mole collagen	0.17 ± 0.02 ^b		0.20 ± 0.005 ^{ab}	0.18 ± 0.01 ^b	0.21 ± 0.01 ^{ab}	0.22 ± 0.003 ^{ab}
moles x-links / mole collagen	0.27 ± 0.03 ^c		0.34 ± 0.01 ^{abc}	0.31 ± 0.02 ^{bc}	0.39 ± 0.01 ^{ab}	0.42 ± 0.03 ^a
Total µg Collagen / mm ³	358.56 ± 33.24		323.23 ± 2.80	350.01 ± 10.60	318.99 ± 14.93	336.10 ± 7.44
µg P / mg dry wt	236.77 ± 3.28		240.49 ± 3.57	244.28 ± 6.04	251.74 ± 1.75	254.18 ± 3.33
Total µg P / mm ³	403.29 ± 11.04 ^c		423.97 ± 6.49 ^{bc}	459.71 ± 20.70 ^{abc}	459.44 ± 10.67 ^{abc}	480.70 ± 8.83 ^{ab}
µg Ca ⁺⁺ / mg dry wt	253.52 ± 32.84		236.40 ± 7.96	211.46 ± 17.28	224.86 ± 8.29	203.67 ± 7.94
Total µg Ca ⁺⁺ / mm ³	424.86 ± 44.25		416.79 ± 14.20	397.82 ± 35.37	410.56 ± 18.89	384.95 ± 14.69
Total µg Ca ⁺⁺ / Total µg Collagen	1.21 ± 0.14		1.29 ± 0.05	1.14 ± 0.11	1.29 ± 0.06	1.15 ± 0.05
Ca ⁺⁺ to P weight ratio	1.07 ± 0.13		0.98 ± 0.04	0.87 ± 0.07	0.89 ± 0.03	0.80 ± 0.04
Total mineral (µg/mm ³)	828.15 ± 39.11		840.76 ± 14.49	857.53 ± 48.95	870.01 ± 26.10	865.65 ± 17.11

Values are means ± standard error. Groups which do not share a similar letter were found significantly different at p<0.05.

Table 3

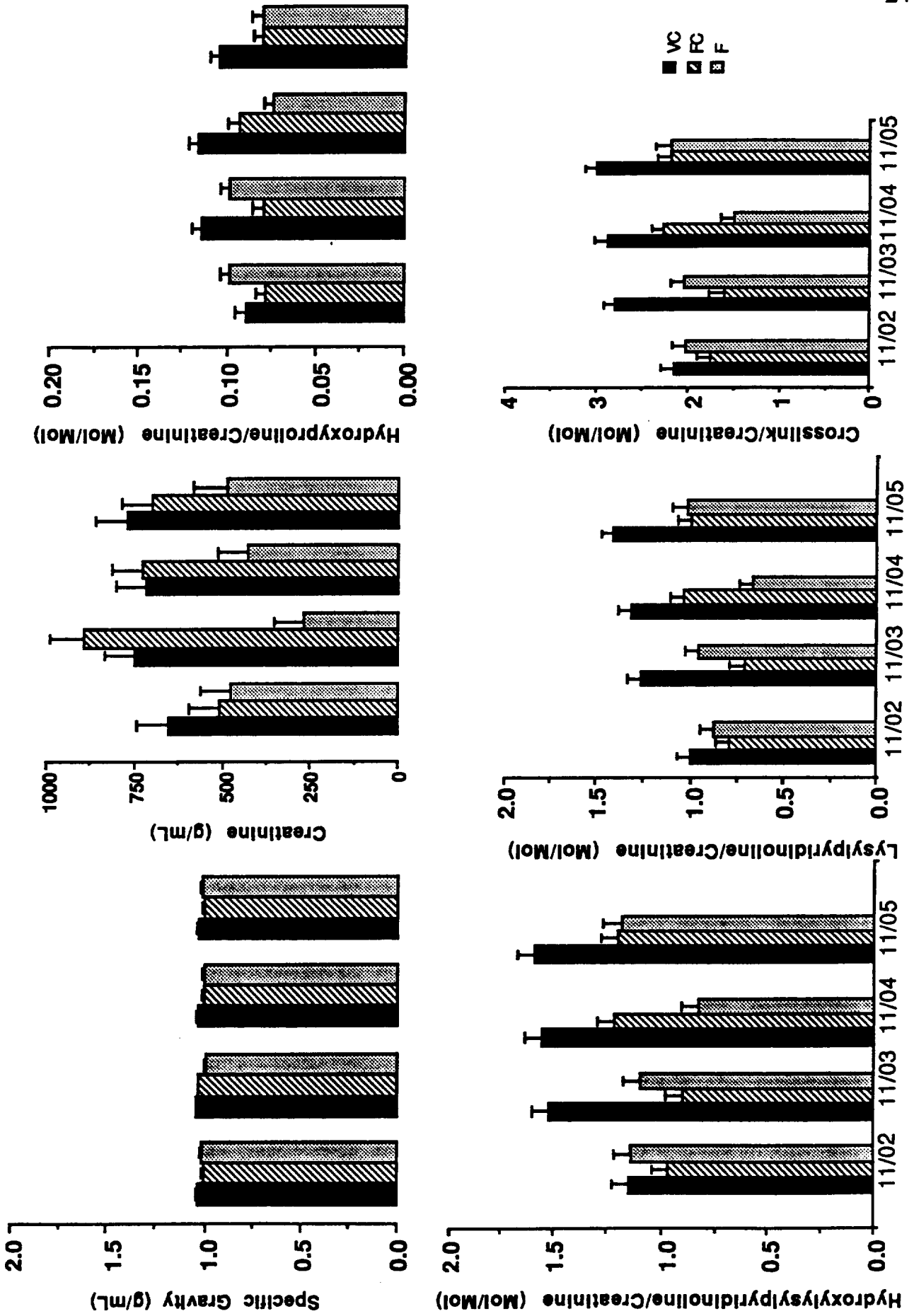
Collagen accumulation

	R+ML		
	VC	FC	F
HUMERUS			
Specific Activity (dpm/ μ Mol HYP)	811.797 \pm 266.492	777.453 \pm 99.438	731.047 \pm 149.291
% New Collagen	(7.73 \pm 0.64) $\times 10^{-7}$	(8.22 \pm 0.43) $\times 10^{-7}$	(8.58 \pm 1.63) $\times 10^{-7}$
% Old Collagen	99.99 \pm 0	99.99 \pm 0	99.99 \pm 0
TIBIA			
Specific Activity (dpm/ μ Mol HYP)	549.320 \pm 378.242	617.816 \pm 105.992	651.456 \pm 246.737
% New Collagen	(5.23 \pm 1.80) $\times 10^{-7}$	(5.88 \pm 0.38) $\times 10^{-7}$	(6.20 \pm 1.05) $\times 10^{-7}$
% Old Collagen	99.99 \pm 0	99.99 \pm 0	99.99 \pm 0

Values are means \pm standard error. No significant differences were found at $p < 0.05$.

SLS-2 Urine

Figure 1



DISCUSSION

The purpose of this study was to examine the residual effects in a growing animal during recovery from a microgravity environment. Second, this study examined the effects of caging upon the growth and maturation of the tibia and humerus in order for recommendations to be made in the implementation of suitable housing modules for future spaceflight research.

In the present study, unlike previous spaceflight studies examining bone metabolism, the turnover of collagen was not a factor influencing bone growth nor the biomechanical attributes associated with the tissue. Bone turnover, the ratio of new to old collagen, did not differ between treatment groups at R+ML. In fact, collagen concentration, nor the total collagen per mm³, accounting for differences in porosity, showed differences between any treatment groups or time points throughout this study. This is in contrast to some of the previous spaceflight studies of shorter duration which examined collagen in the tibia and humerus (Martinez, *et al.*, 1988; personal communication, Dr. Emily M. Holton, NASA Ames Research Center, CA, 1994). However, Vailas, *et al.*, (1992) also found no change in collagen with 14 d spaceflight. Although, the present study utilized 38 d old rats which were younger than rodents used in previous studies designed to describe the effects of a microgravity environment on bone. Previous spaceflight studies involved adult rats beyond the period in development denoted by rapid bone growth (Hansson, *et al.*, 1972).

Bone growth, indicated by length and total cross-sectional area, significantly increased in 28 d (Appendix A). These parameters showed no change in 14 d of recovery, however, except in the cross-sectional area of the humerus. An unexpected contradiction to the decrease in skeletal growth was that these rodents continued to show an increase in body weight for the duration of the study (Appendix B). The increase in body weight, therefore, may reflect an increase in the mass of non-skeletal tissues. These changes in growth were similar to those found in the tibia of 65 to 81 d old rodents flown aboard SLS-1 for 9 d (personal communication, Dr. Emily M. Holton, NASA Ames Research Center, CA, 1991). SLS-1 groups differed in length and cross-sectional area after 16 d growth from launch to R+ML. The R+O F group in SLS-1, however, unlike that

found in SLS-2, showed significantly less bone growth than the R+ML rodents which were 1 wk older. The length of time in flight on SLS-1, however, was only 9 d which was 5 d less than SLS-2. The bone growth in the tibia during these additional 5 d may have resumed to normal or above normal in the SLS-2 study. Both studies involved R+O and R+ML rodents of ages beyond the period of rapid bone growth, illustrating the normal decline in bone growth associated with adulthood, and emphasizing the need to perform spaceflight studies on younger rodents. Further support for the age-related decline in bone growth comes from data collected immediately following a 14 d spaceflight mission aboard COSMOS 2044 from 124 d old rats in which Vailas, *et al.*, (1992) failed to observe longitudinal nor cross-sectional area growth in the humerus. It may be important to note the difference in diet between the NASA and COSMOS missions. COSMOS missions have in the past used a paste diet consisting of 70% water, whereas, the SL-missions in the United States have used food bars (Teklad, L-356) (Montufar-Solis, *et al.*, 1992). This difference in diet along with the competition for food in group housing habitats may help to explain some of the differences observed between COSMOS and SL-mission results.

Biomechanical tests conducted earlier on the same left humerus and tibia used in the present study found increases in structural (size and strength) and material (strength/area) properties with age (Appendix C) (personal communication, Dr. Ray Vanderby, University of Wisconsin-Orthopedics, 1994). These values were significantly greater in animals 14 and 28 d older, despite a lack of change in collagen. Significant increases in the amount of crosslinks per mole of collagen with an additional 14 or 28 d of age supports the biomechanical data outlining increases in stiffness, energy to failure, peak load, deformation at failure, and elastic modulus as the animal grew older. Other factors which were not quantified in the present study may have also influenced the structural and material properties of the tibia and humerus. These factors may include, but are not limited to, the porosity of the tissue (porosity was not measured in the present study, density was used as an indirect measurement), the distribution of minerals within the bone which have been shown by Mechanic, *et al.*, (1990) to be affected by 12.5 d spaceflight, and mineral crystal size.

It is interesting to note that there were no appreciable changes in mineral concentration in the tissue as a result of spaceflight or an increase in biological age. It has been hypothesized by Klein, *et al.*, (1985) and Yamauchi and Mechanic (1988) that collagen maturation through crosslinking and mineralization may be interrelated and somewhat dependent upon one another. However, the present study is unique in that it provides evidence against this hypothesis. There were no appreciable increases in mineralization with advancing age, despite increases in crosslinks in 14 and 28 d older rats. Therefore, unlike the hypothesis proposed by Klein, *et al.*, (1985) and Yamauchi and Mechanic (1988), which states that with increases in crosslinking, mineralization will be inhibited, the results from the present study suggest that increased crosslinking within a given range may have minimal effects on the degree of mineralization. This may not be the case, however, with further increases in crosslink concentration which may interfere with the mineralization process through stereochemical inhibition.

There were no effects of flight on collagen metabolism, maturation, or mineralization except in the tibia where there were greater amounts of LP crosslinks per mole of collagen in the F versus FC group. There was also a trend towards a decreased concentration of collagen in the tibia F versus FC group. This relationship might suggest that in these young animals protein synthesis of collagen decreases with flight, resulting in an increase in the number of crosslinks per mole of collagen present. Spaceflight may effect collagen maturation in this manner in young, growing animals, however, further studies are necessary to definitively identify this adaptation to microgravity.

There are at least three important changes to note from the urine data. First, flight and the smaller cage size may have a suppressive effect on the resorption of mature collagen. Changes in the resorption of mature collagen seem to occur to a new level of homeostasis or steady state, which is lower than that found in the VC group. After 4 d following the landing of the shuttle, the FC and F groups are no longer significantly different from one another. Second, a suppression of protein synthesis and/or decreased resorption of mature collagen leads one to expect either no change or an increase in the amount of crosslinks in the tissue. This is precisely what is found. In

the tissue, there were no significant differences in collagen between groups at any of the time points, however, crosslinks increased with age, and in the tibia the LP crosslinks were significantly greater in the F group. Whereas, the urinary crosslinks exhibited a decreased resorption with flight and smaller cage size. It is possible, therefore, that animals attempt to adapt to microgravity and hypokinetic environments through some unknown control mechanisms regulating the protein synthesis of collagen and the resorption of mature collagen from the weightbearing skeleton. Future investigations should focus upon the regulation of protein synthesis at the mRNA level to gain further insight into the control mechanisms involved. Third, mature collagen resorption in the VC group continued to increase throughout the four days of urine collection while there was no change or an increase in mature collagen in the tissue. This finding suggests that there was an increase in the production of mature collagen in bone which compensated for the increased loss in urine. This high rate of synthesis and resorption may be important to obtaining a structurally intact tissue able to resist biomechanical stresses imposed upon it, however, no differences were observed in the structural and material attributes measured between treatment groups at R+O or R+ML. It is important when evaluating changes in these urinary markers to rely on urinary crosslinks as an indicator of mature collagen resorption and not on urinary HYP. Error in urinary HYP measurements and the interpretation of them may be due at least in part to the capacity of the liver to convert free HYP to pyrroline-3-hydroxy-carboxylic acid by the enzyme hydroxyproline oxidase (Cundy, *et al.*, 1983; Uebelhart, *et al.*, 1990). The urinary HYP results, therefore, may not accurately reflect the degradation of collagen in the tissue, because it may be influenced by the ability of the liver to remove HYP. The urine data also indicate an obvious fluctuation in the metabolism of collagen during the first 4 d postflight. Future experiments should attempt to quantify the amount of new and old collagen in these animals at 4 d postflight to identify differences in steady states between both 4 d and 14 d of recovery.

The F group experienced a suppressed glomerular filtration rate (GFR) during the first 4 d of recovery which began to increase toward normal levels during days 3 and 4 of urine collection. Creatinine is used as a physiological indicator of GFR because it is filtered almost completely by

the renal tubules. Another substance sometimes used to measure the GFR is inulin. Inulin is a synthetic substance which must be injected into the body and is then completely filtered at the kidney into the urine. Since inulin was not provided to these rodents, however, creatinine, a by-product of creatine produced in muscle, was used. The use of this marker, however, is difficult because it is not completely filtered like inulin and its production increases with muscle atrophy or damage. Decreases in skeletal muscle mass and fiber cross-sectional areas occur, especially in slow-twitch muscles, with spaceflight. Whereas, the percentage of fast-twitch fibers normally increases during spaceflight (Grindeland, *et al.*, 1992). By normalizing the HYP and crosslink data to creatinine, however, an accurate assessment of changes in resorption is still made possible.

No appreciable caging effects were observed in the tissue. The lack of significant differences observed between FC and VC in both the humerus and tibia in the variables quantitated suggest that both size habitats serve as suitable controls. These results agree with other data describing a lack of observable change in the composition and metabolism of bone with inactivity. This is an important finding demonstrating that comparisons between COSMOS studies utilizing the VC cages housing multiple animals per cage aboard the shuttle and NASA spaceflights which employ the smaller, FC cages housing animals individually, are acceptable and will not significantly impact collagen metabolism, maturation, or mineralization in the tibia or humerus.

The important conclusions as a result of this spaceflight study used to investigate bone metabolism following a 2 wk period in a microgravity environment are as follows: First, this study supports the hypothesis that crosslinking increases with age and that crosslinking is independent of mineralization in the tibia and humerus. Second, contrary to previous results from our laboratory, there were no effects of spaceflight except for an increase in nonreducible LP crosslinks per mole of collagen in the tibia, indicating that spaceflight had an effect on collagen maturation in these animals. Third, despite a continued increase in body weight over time, there were no appreciable increases in skeletal growth during the recovery period. Fourth, a reduced excretion of mature collagen suggests a downregulation of resorption in FC and F animals. Finally, there were no caging effects impacting tissue growth and maturation in the tissue. This

finding suggests that both vivarium size cages, housing a large number of rodents, and flight control cages of a slightly smaller size, housing rodents individually, are both suitable control models for spaceflight research. In order for additional information to be obtained concerning the changes in bone metabolism with spaceflight and the mechanisms of control processes, it is recommended that future spaceflight studies include younger rodents and radioactive labeling at L+O in an attempt to describe the adaptations during growth in a microgravity environment in more detail.

Appendix A

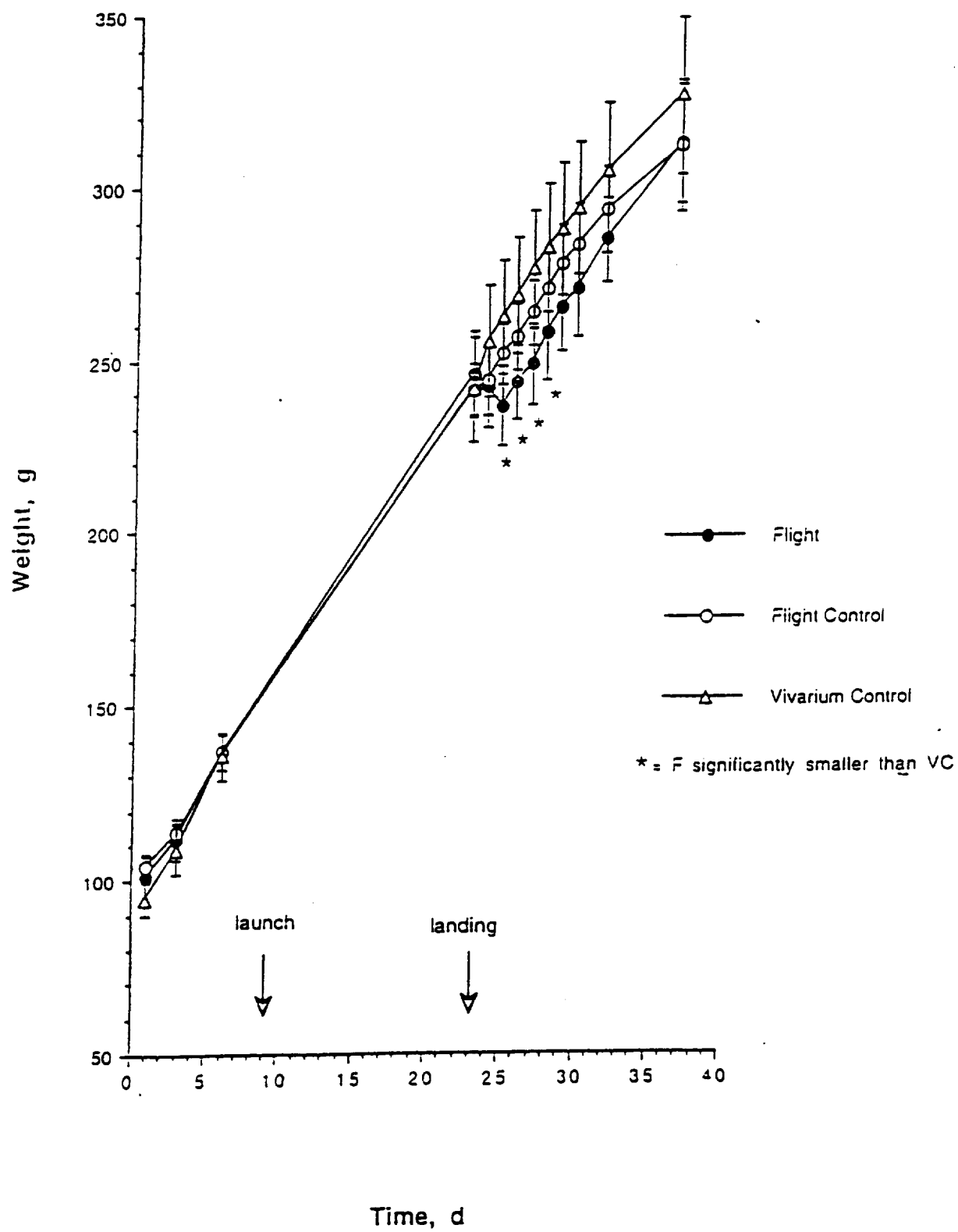
SLS-2 Tissue Growth

	L+O		R+O		R+ML	
	Basal	Viv. Control	Flight Control	Flight	Viv. Control	Flight Control
HUMERUS						
Length (mm)	21.215 ± 0.927 ^b	23.781 ± 0.594 ^a	24.127 ± 0.074 ^a	23.830 ± 0.617 ^a	26.459 ± 0.131 ^a	26.362 ± 0.177 ^a
Total Cross-Sectional Area (mm ²)	3.945 ± 0.068 ^c	4.852 ± 0.093 ^b	4.735 ± 0.073 ^b	5.066 ± 0.091 ^b	5.269 ± 0.181 ^a	5.174 ± 0.175 ^a
TIBIA						
Length (mm)	30.493 ± 0.303 ^c	32.989 ± 1.393 ^{ab}	34.204 ± 0.800 ^b	35.229 ± 0.243 ^{ab}	37.826 ± 0.291 ^a	37.577 ± 0.297 ^a
Total Cross-Sectional Area (mm ²)	4.346 ± 0.127 ± ^b	4.926 ± 0.205 ^{ab}	5.010 ± 0.283 ^{ab}	4.659 ± 0.224 ^{ab}	5.291 ± 0.199 ^a	5.046 ± 0.097 ^{ab}

Values are means ± standard deviation.

Groups sharing the same letter are not significantly different from one another at $p < 0.05$.

SLS2 RAT GROWTH



SLS-2 Biomechanical Attributes

	L+O		R+O		R+ML	
	Basal	Viv. Control	Flight Control	Flight	Viv. Control	Flight Control
HUMERUS						
Stiffness (N/mm)	41.62 ± 16.22 ^a	76.85 ± 19.31 ^{af}	67.72 ± 13.64 ^{af}	67.38 ± 23.44 ^f	119.5 ± 22.7 ^{af}	124.2 ± 14.3 ^{af}
Energy to Failure (N-mm)	15.43 ± 2.26 ^b	19.58 ± 3.21 ^f	19.48 ± 3.75 ^f	18.92 ± 4.58 ^f	21.38 ± 12.82 ^{bf}	28.18 ± 7.12 ^{bf}
Peak Load (N)	29.52 ± 3.11 ^c	45.45 ± 4.13 ^{cf}	41.92 ± 4.94 ^{cf}	39.12 ± 6.96 ^{cf}	63.03 ± 10.20 ^{cf}	70.42 ± 2.78 ^{cf}
Deformation at Failure (mm)	0.903 ± 0.207 ^d	0.690 ± 0.076 ^d	0.748 ± 0.109	0.772 ± 0.104	0.688 ± 0.099	0.692 ± 0.108 ^d
Elastic Modulus (N/mm ²)	1687 ± 539 ^e	2103 ± 692	1846 ± 386 ^f	1839 ± 639 ^g	2532 ± 698	2787 ± 625 ^{ef}
TIBIA						
Stiffness (N/mm)	44.23 ± 1.64 ^c	74.10 ± 10.55 ^{ef}	61.60 ± 10.29 ^{ef}	64.40 ± 6.63 ^{cf}	141.7 ± 34.9 ^{ef}	157.8 ± 27.0 ^{ef}
Energy to Failure (N-mm)	26.72 ± 5.68 ^c	46.06 ± 2.93 ^c	43.02 ± 10.34 ^c	41.65 ± 5.94 ^c	45.72 ± 5.66 ^c	43.11 ± 7.74 ^c
Peak Load (N)	39.80 ± 3.43 ^c	68.92 ± 2.55 ^{ef}	63.70 ± 9.14 ^{cf}	64.53 ± 3.88 ^{cf}	94.42 ± 11.10 ^{ef}	97.29 ± 12.10 ^{ef}
Deformation at Failure (mm)	1.127 ± 0.132 ^b	1.278 ± 0.052 ^f	1.263 ± 0.169 ^f	1.280 ± 0.109 ^f	0.866 ± 0.215 ^{bf}	0.790 ± 0.123 ^{bf}
Elastic Modulus (N/mm ²)	1485 ± 202 ^b	1724 ± 487 ^f	1518 ± 588 ^f	1762 ± 279 ^f	2752 ± 554 ^{bf}	3370 ± 601 ^{bf}

Values are means ± standard deviation. Significance level $p < 0.05$.

^a L+O basal group is significantly different than all other groups except R+O F.

^b L+O basal group is significantly different than all R+ML groups.

^c L+O basal group is significantly different than all other groups.

^d L+O basal group is significantly different than R+O VC, R+ML FC and F groups.

^e L+O basal group is significantly different than R+ML FC and F groups.

^f R+O group is significantly different than R+ML group.

^g R+O FC group is significantly different than the R+O VC group.

Appendix D

Calculations for Collagen Concentration in Bone Samples

Known or Measured Information

HYP area of sample from HPLC

100 pMoles = standard area from HPLC

Aliquots and volumes used

Molecular weight of collagen = 300000 g / mole

Molecular weight of HYP = 131.13 g / mole

Collagen to HYP weight ratio = 7 to 1

Calculations

mMoles HYP loaded on column = (sample area) * (100 pMoles / standard area) * (1 mMole / 1×10^9 pMoles)

mMoles HYP / mL = (# mMoles loaded) * (hydrolyzate volume / aliquot for HYP) * (volume resuspended / aliquot for sep pak) * (volume resuspended / aliquot injected)

Total # mMoles collagen / mL = (# mMoles HYP / mL) * (131.13 mg HYP / 1 mMole HYP) * (7 mg collagen / 1 mg HYP) * (1 mMole collagen / 300000 mg collagen)

Appendix E

Calculations for % New and Old Collagen Present in Bone

Known or Measured Information

Molecular weight of collagen = 300000 g / mole

Molecular weight of HYP = 131.13 g / mole

Collagen to HYP weight ratio = 7 to 1

1 μCi = 2.22×10^6 dpm

Total # mMoles collagen / mL

^3H -CPM from scintillation counter from sample

background # ^3H -CPM from scintillation counter from blank

Counting efficiency of scintillation counter = 60.3%

Source injected = 43 Ci / mMole proline = 4.3×10^7 μCi / mMole proline

mMoles HYP / mL = # mMoles proline / mL

Calculations

DPM Corrected / mL = (((# ^3H -CPM from sample) - (background # CPM)) / counting efficiency) * (1 mL / 0.9 mL aliquot)

μCi / mL = (DPM Corrected / mL) * (1 μCi / 2.2×10^6 dpm)

mMoles proline / mL = (1 mMole proline / 4.3×10^7 μCi) * (# μCi / mL)

mMoles new collagen / mL = (# mMoles HYP / mL) * (131.13 mg HYP / mMole HYP) * (7 mg collagen / mg HYP) * (1 mMole collagen / 300000 mg collagen)

mMoles old collagen / mL = (Total # mMoles collagen / mL) - (# mMoles new collagen / mL)

% New Collagen = (# mMoles new collagen / mL) / (Total # mMoles collagen / mL)

% Old Collagen = (# mMoles old collagen / mL) / (Total # mMoles collagen / mL)

Urine Biochemistry

	Specific Gravity (g/mL) ^x	Creatinine (μg/mL)	HYP/Cr (Mol/Mol)	HP/Cr (Mol/Mol) x 10 ⁻⁴	LP/Cr (Mol/Mol) x 10 ⁻⁴	X-link/Cr (Mol/Mol) x 10 ⁻⁴
VC	1.027 ± 0.006 ^{uyGOS}	654.997 ± 86.990 ^u	0.0895 ± 0.0056 ^{l2}	1.15 ± 0.081 ^{23ky}	0.99 ± 0.071 ^{23yGK}	2.14 ± 0.141 ^{23yK}
	1.040 ± 0.006 ^{rvzHDPT}	747.670 ± 86.990 ^{rvz}	0.1137 ± 0.0056 ^{l2DHLPT}	1.52 ± 0.081 ^{rvzDHLPT}	1.26 ± 0.071 ^{rvzDHLPT}	2.78 ± 0.141 ^{rvzDHLPT}
	1.028 ± 0.006 ^{uwAIQ}	715.045 ± 86.990 ^{uA}	0.1162 ± 0.0056 ^{2nuwAEIQU}	1.56 ± 0.082 ^{awAEIQU}	1.31 ± 0.072 ^{awAEIQU}	2.88 ± 0.142 ^{awAEIQU}
	1.027 ± 0.006 ^{zBJRV}	772.122 ± 86.990 ^{zBFJ}	0.1045 ± 0.0056 ^{BFJNV}	1.59 ± 0.083 ^{zBFJNRV}	1.41 ± 0.073 ^{zBFJNRV}	3.00 ± 0.143 ^{zBFJNRV}
FC	1.007 ± 0.006 ^{IGHU}	505.590 ± 86.990 ^{l1}	0.0780 ± 0.0056 ^{teHD}	0.96 ± 0.082 ^{3HD}	0.79 ± 0.072 ^{mGHU}	1.75 ± 0.142 ^{3mGHU}
	1.025 ± 0.007 ^{lfj}	894.435 ± 97.904 ^{lbfjn}	0.0795 ± 0.0064 ^{bfLMN}	0.89 ± 0.094 ^{5bmKLMN}	0.71 ± 0.084 ^{5bmKLMN}	1.60 ± 0.164 ^{5bmKLMN}
	1.008 ± 0.006 ^{OPQR}	727.748 ± 86.990 ^{gk}	0.0930 ± 0.0056 ^{gkPQ}	1.22 ± 0.082 ^{4EPQR}	1.04 ± 0.072 ^{4EPQR}	2.26 ± 0.142 ^{4EPQR}
	1.008 ± 0.006 ^{STUV}	701.680 ± 86.990 ^{hl}	0.0803 ± 0.0056 ^{dHTUV}	1.20 ± 0.083 ^{5ITTUV}	0.99 ± 0.075 ^{ITTUV}	2.19 ± 0.143 ^{5ITTUV}
F	1.012 ± 0.006 ^f	477.083 ± 86.990 ^{bert}	0.0982 ± 0.0056 ^{23abds}	1.14 ± 0.082 ^{brst}	0.87 ± 0.072 ^{zrst}	2.01 ± 0.142 ^{zrst}
	1.000 ± 0.006 ^{fuvwx}	265.950 ± 86.990 ^{fghuvwx}	0.0980 ± 0.0056 ^{45efhw}	1.09 ± 0.082 ^{4vwx}	0.95 ± 0.074 ^{fvwx}	2.04 ± 0.144 ^{fvwx}
	1.003 ± 0.006 ^{yzAB}	427.892 ± 86.990 ^{jkizAB}	0.0735 ± 0.0056 ^{24kzAB}	0.83 ± 0.084 ^{6klyzAB}	0.67 ± 0.072 ^{46klyzAB}	1.50 ± 0.142 ^{46klyzAB}
	1.014 ± 0.007 ^D	484.816 ± 97.904 ^{nf}	0.0800 ± 0.0064 ^{35DEF}	1.18 ± 0.096 ^{nDEF}	1.01 ± 0.086 ^{mnDEF}	2.19 ± 0.166 ^{mnDEF}

Urine collected in a.m. during R+O + 1, 2, 3, and 4 days. Values are mean ± standard error. Statistical significance at p<0.05.

- ¹ 11/02 is significantly different than 11/03 within the same treatment group.
- ² 11/02 is significantly different than 11/04 within the same treatment group.
- ³ 11/02 is significantly different than 11/05 within the same treatment group.
- ⁴ 11/03 is significantly different than 11/04 within the same treatment group.
- ⁵ 11/03 is significantly different than 11/05 within the same treatment group.
- ⁶ 11/04 is significantly different than 11/05 within the same treatment group.

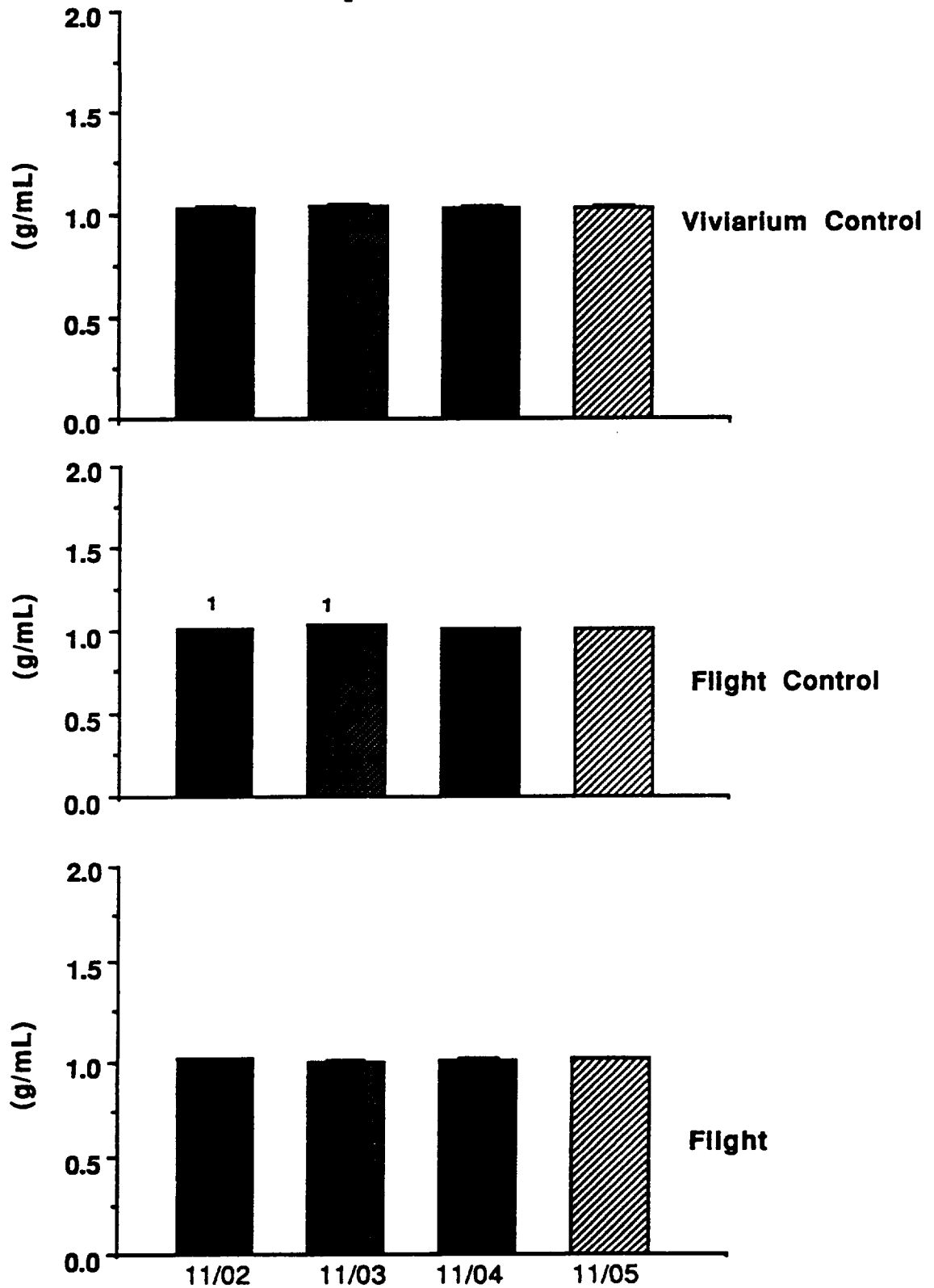
Appendix G

Appendix F Significance Key Continued

- a F 11/02 is significantly different than FC 11/02.
- b F 11/02 is significantly different than FC 11/03.
- c F 11/02 is significantly different than FC 11/04.
- d F 11/02 is significantly different than FC 11/05.
- e F 11/03 is significantly different than FC 11/02.
- f F 11/03 is significantly different than FC 11/03.
- g F 11/03 is significantly different than FC 11/04.
- h F 11/03 is significantly different than FC 11/05.
- i F 11/04 is significantly different than FC 11/02.
- j F 11/04 is significantly different than FC 11/03.
- k F 11/04 is significantly different than FC 11/04.
- l F 11/04 is significantly different than FC 11/05.
- m F 11/05 is significantly different than FC 11/02.
- n F 11/05 is significantly different than FC 11/03.
- o F 11/05 is significantly different than FC 11/04.
- p F 11/05 is significantly different than FC 11/05.
- q F 11/02 is significantly different than VC 11/02.
- r F 11/02 is significantly different than VC 11/03.
- s F 11/02 is significantly different than VC 11/04.
- t F 11/02 is significantly different than VC 11/05.
- u F 11/03 is significantly different than VC 11/02.
- v F 11/03 is significantly different than VC 11/03.
- w F 11/03 is significantly different than VC 11/04.
- x F 11/03 is significantly different than VC 11/05.
- y F 11/04 is significantly different than VC 11/02.
- z F 11/04 is significantly different than VC 11/03.

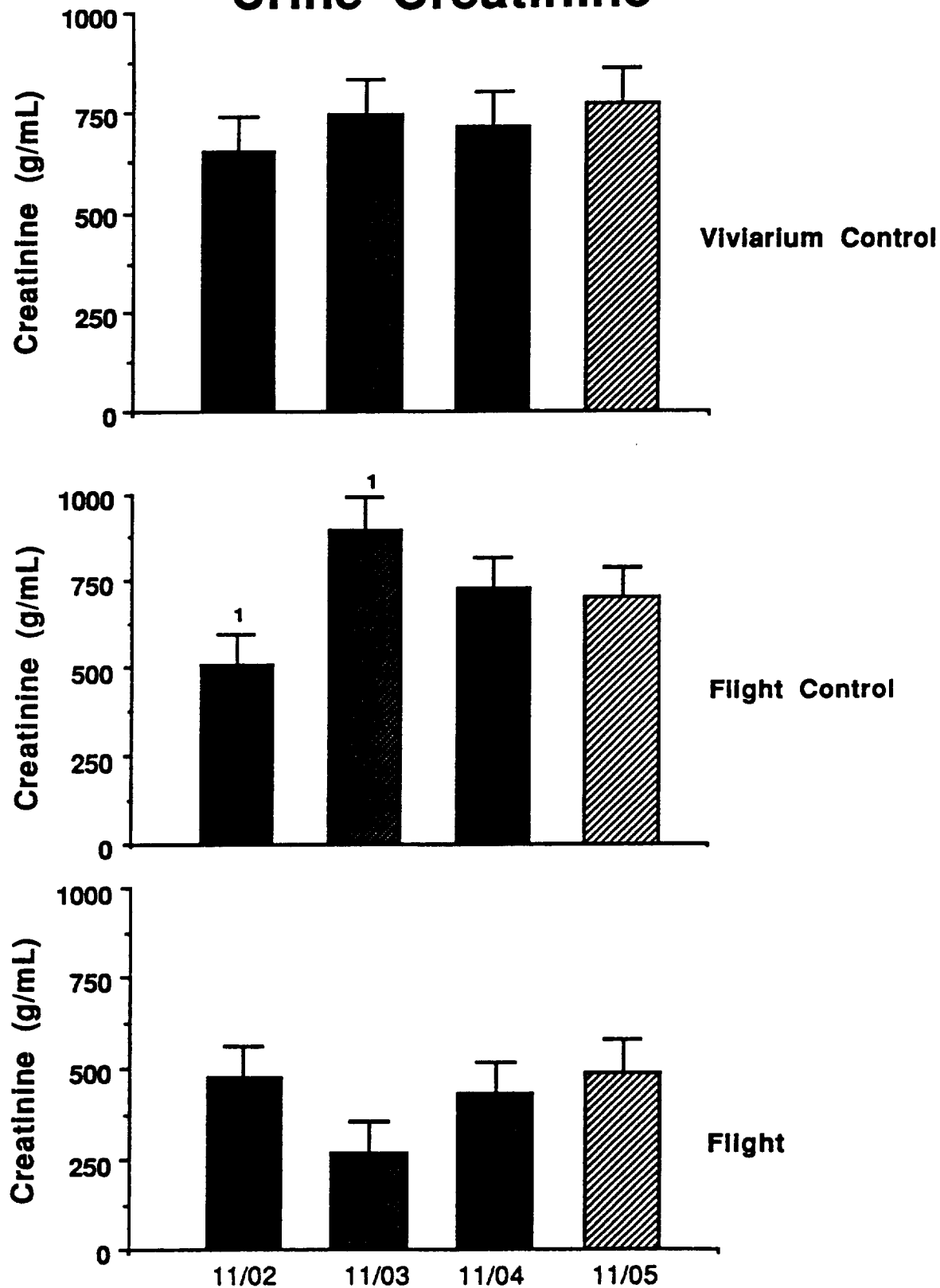
- A F 11/04 is significantly different than VC 11/04.
- B F 11/04 is significantly different than VC 11/05.
- C F 11/05 is significantly different than VC 11/02.
- D F 11/05 is significantly different than VC 11/03.
- E F 11/05 is significantly different than VC 11/04.
- F F 11/05 is significantly different than VC 11/05.
- G FC 11/02 is significantly different than VC 11/02.
- H FC 11/02 is significantly different than VC 11/03.
- I FC 11/02 is significantly different than VC 11/04.
- J FC 11/02 is significantly different than VC 11/05.
- K FC 11/03 is significantly different than VC 11/02.
- L FC 11/03 is significantly different than VC 11/03.
- M FC 11/03 is significantly different than VC 11/04.
- N FC 11/03 is significantly different than VC 11/05.
- O FC 11/04 is significantly different than VC 11/02.
- P FC 11/04 is significantly different than VC 11/03.
- Q FC 11/04 is significantly different than VC 11/04.
- R FC 11/04 is significantly different than VC 11/05.
- S FC 11/05 is significantly different than VC 11/02.
- T FC 11/05 is significantly different than VC 11/03.
- U FC 11/05 is significantly different than VC 11/04.
- V FC 11/05 is significantly different than VC 11/05.
- W FC is significantly different than VC.
- X F is significantly different than VC.

Urine Specific Gravity

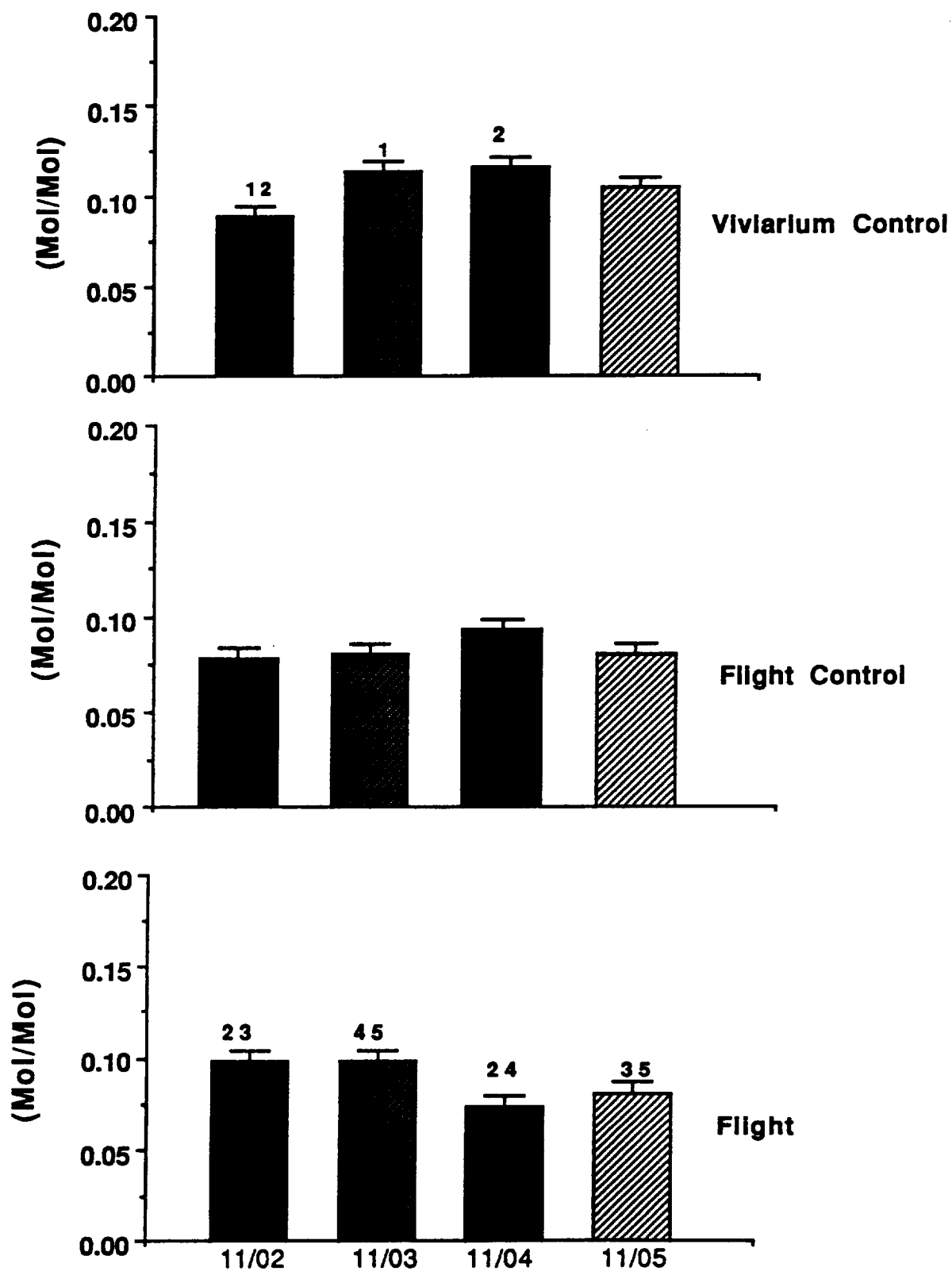


Groups sharing similar numbers are significantly different at $p < 0.05$.

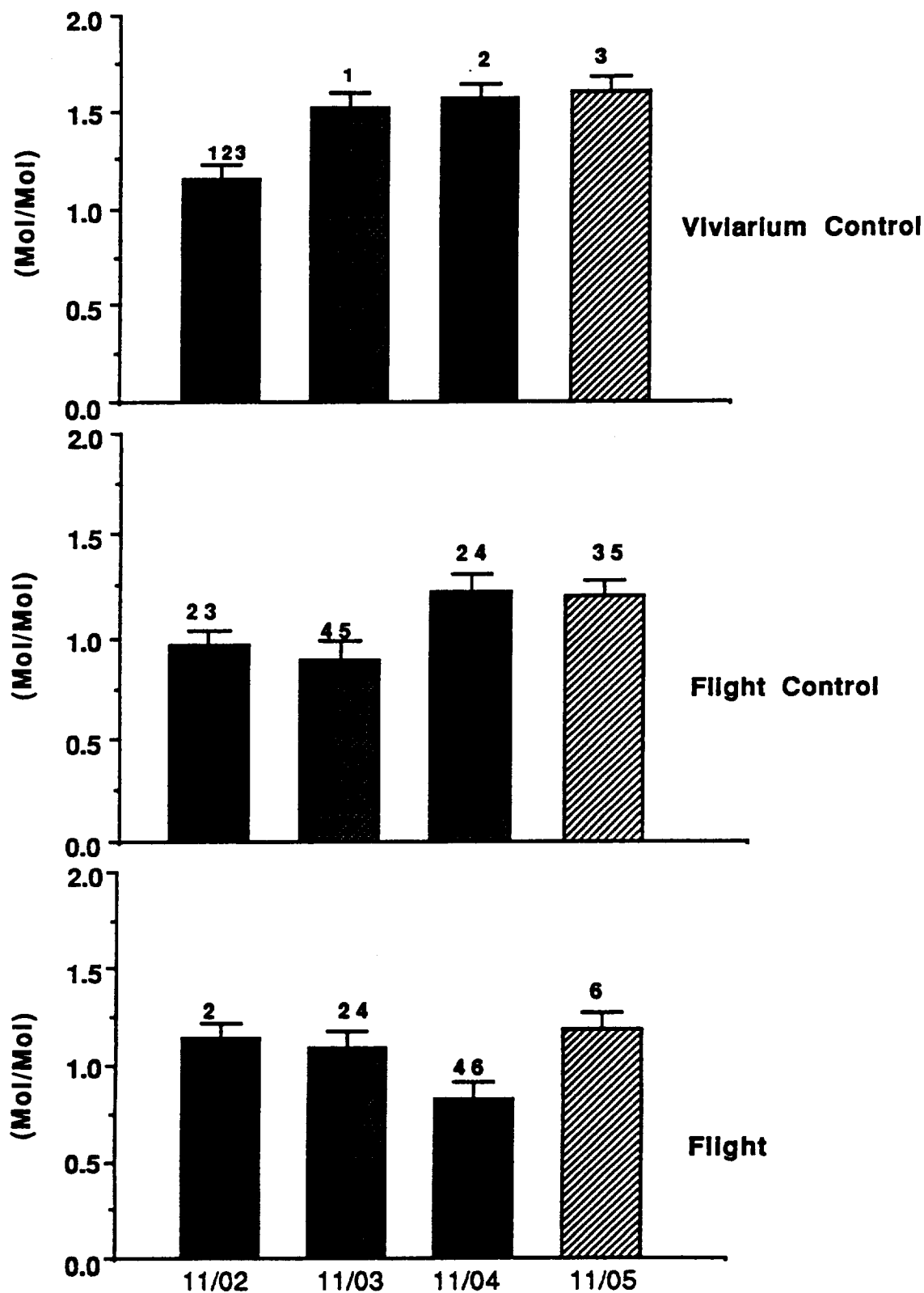
Urine Creatinine



Groups sharing similar numbers are significantly different at $p < 0.05$.

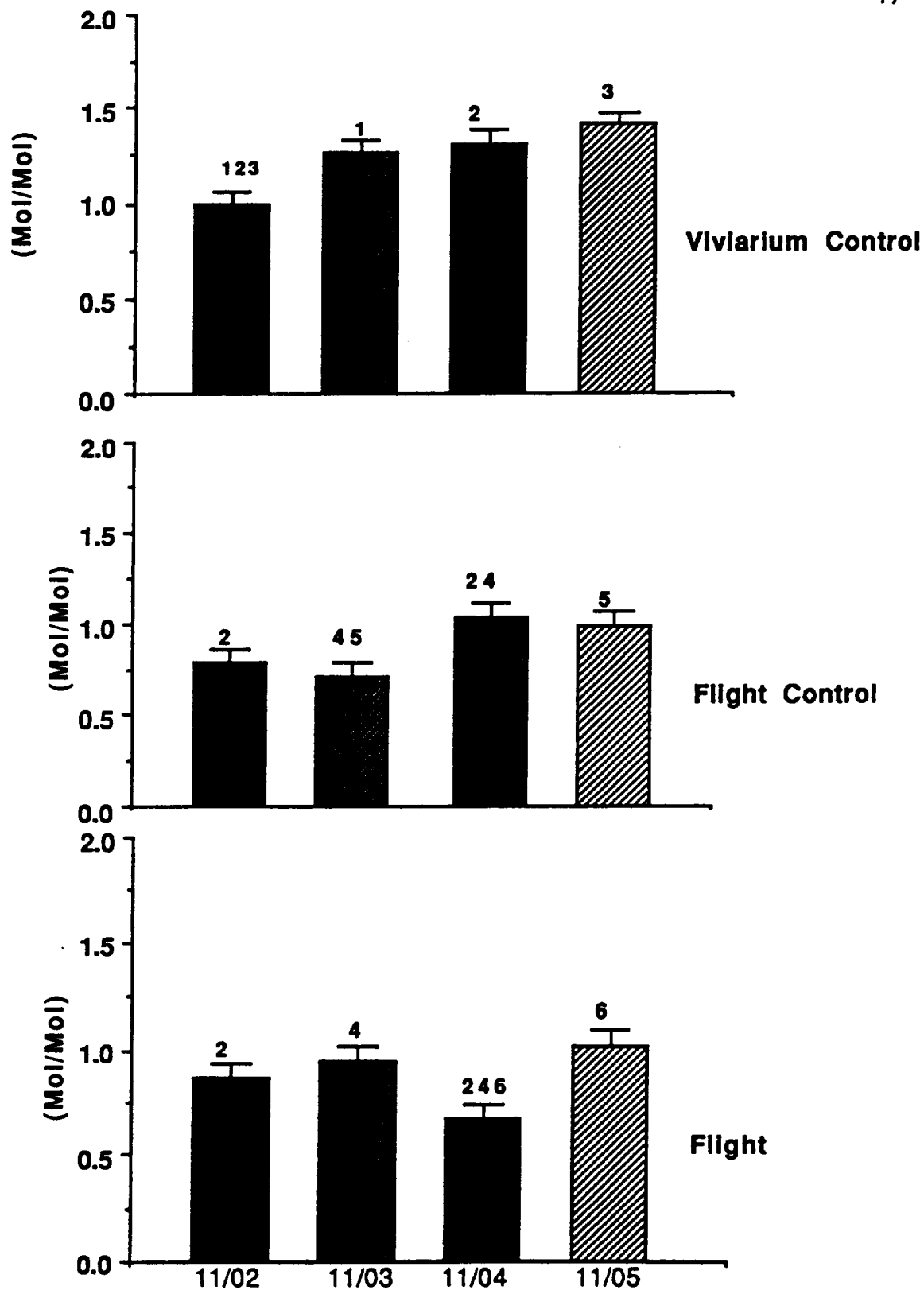


Groups sharing similar numbers are significantly different at $p < 0.05$.



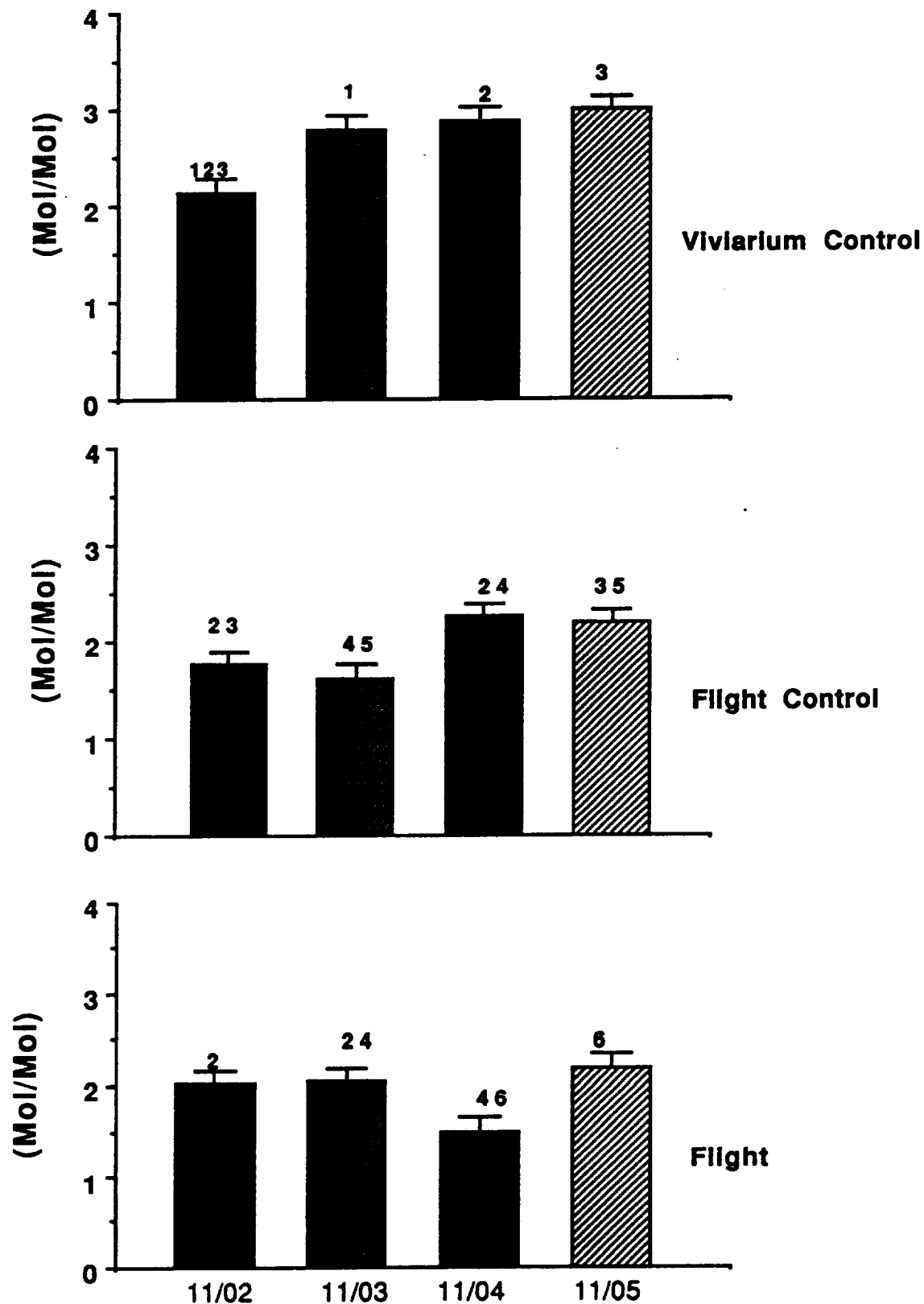
Groups sharing similar numbers are significantly different at $p < 0.05$.

Urine LP/Cr



Groups sharing similar numbers are significantly different at $p < 0.05$.

Urine X-link/Cr



Groups sharing similar numbers are significantly different at $p < 0.05$.